

Effectiveness of Lontar Sap Vinegar and Lime Juice as a Substitute for HCl in the Production of Skipjack Fish Bone Gelatin

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Article History:

Received : 28 October 2024

Revised : 07 January 2025

Accepted : 17 January 2025

Keywords:

Acid concentration,
Gelatin skipjack fish bones,
Lime juice extract,
Palm sap vinegar.

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ABSTRACT

*Gelatin is a product of collagen hydrolysis that is widely used in the food, pharmaceutical, and cosmetic industries. The gelatin production process generally uses hydrochloric acid (HCl) as a hydrolysis agent, but its use raises concerns about the environment and food safety. This study aims to evaluate the effectiveness of palm sap vinegar and lime juice extract as a substitute for HCl in the production of gelatin from skipjack fish bones (*Katsuwonus pelamis*). The study used a 3 x 3 factorial design with two main factors, namely the type of acid compound (HCl, coconut sap vinegar, and lime juice extract) and the acid concentration (5%, 7%, and 9%). The variables observed included yield, pH, moisture content, ash content, fat content, and gelatin protein content. The results showed that the type of acid and the concentration of acid significantly affected the quality of gelatin. Palm sap vinegar produces gelatin with characteristics almost equivalent to HCl, with the highest protein content of 53.77%. Meanwhile, lime juice extract produced lower protein content (48.87%) yet provided lower fat content. The 7% and 9% acid concentration produces gelatin of the highest quality in terms of yield and protein content. This study shows that palm sap vinegar can be environmentally friendly and effective alternatives to replace HCl in gelatin production, with some adjustments to their concentration and application.*

1. INTRODUCTION

Gelatin is one of the products that has high economic value and is widely used in various industries, such as food, pharmaceuticals, and cosmetics. In the food industry, gelatin functions as a thickening agent, stabilizer, emulsifier, and gel former. In the pharmaceutical sector, gelatin is used as a capsule material, while in the cosmetics industry, gelatin is used as a basic ingredient in making skin care products. Due to its various uses, the demand for gelatin in the market continues to increase every year. However, most of the gelatin produced commercially comes from the bones and skin of animals such as cows and pigs, which is a challenge especially in countries with a Muslim majority population that require halal gelatin (Arnmalia *et al.*, 2021; Prasetyo *et al.* (2023).

Fish is one of the alternative sources of gelatin that is starting to be widely studied, especially fish bones which are a by-product of the fish processing industry. Fish bones, which have long been considered waste (Alhana *et al.*, 2015), are actually rich in collagen, a protein that can be hydrolyzed into gelatin. One of the fish species that has the potential to be used as a source of gelatin is skipjack tuna (*Katsuwonus pelamis*), which is widely found in Indonesian waters (Bora *et al.*, 2022). Skipjack tuna bones contain collagen with a yield of around 16.17% of the weight of the fish bones, so they have the potential to be processed into good quality gelatin (Rodiah *et al.*, 2018; Sutardi *et al.*, 2020; Septiansyah *et al.*, 2020). In addition, another study by Singkuku *et al.* (2017), showed that gelatin extracted from

skipjack tuna bones has a protein content ranging from 17.6–58.2%, indicating the potential of skipjack tuna bones as a good source of collagen.

The bones contain 18.6% collagen and 19.86% complex protein organic elements (Liu *et al.*, 2009). The use of skipjack tuna bones as a source of gelatin not only has the potential to reduce fishery waste, but also adds economic value to fishery by-products (Jalili *et al.*, 2022). The processing of collagen in fish bone waste into gelatin can be done with initial treatment, namely the use of chemical compounds that react with acids or bases (Panjaitan, 2016). Both of these solvents (acid or base) can be used in the manufacture of gelatin (Bennion, 1980).

The process of processing collagen into gelatin production, one of the important stages is soaking the raw fish bone material in an acid solution to hydrolyze the collagen into gelatin (Benion, 1989; Panjaitan, 2016). The most commonly used acid in the production of gelatin that meets SNI standards is hydrochloric acid (HCl) (Alhana *et al.*, 2015; Panjaitan, 2016; Pertiwi *et al.*, 2018; Farida *et al.*, 2020). The use of hydrochloric acid (HCl) in extracting collagen from fish bones is indeed effective, but there are several disadvantages, namely it can reduce the yield and water content of the gelatin produced along with the increasing concentration of HCl (Pamungkas, 2019; Mulyani *et al.*, 2021), the ash content in gelatin can increase and can affect the purity and final quality of the gelatin product ; (Hanivia & Jariah, 2022).

To overcome the disadvantages of using HCl, several studies have tried to use organic acids (natural) as an alternative that is more environmentally friendly and safer for health (Gerold *et al.*, 2022). Some organic acids that have been tested include citric acid, lactic acid, and vinegar. The concentration of organic acids used in gelatin extraction varies between 2% and 9%, depending on the type of raw material and acid used (Pertiwi *et al.*, 2018). Research by Santoso *et al.* (2015) and Pertiwi *et al.* (2018), stated that the use of citric acid and acetic acid with concentrations of 7% and 9% produced gelatin quality that was not significantly different from HCL. The use of this organic acid is expected to produce gelatin with a quality equivalent to the use of HCl (Arshad *et al.*, 2021). In Indonesia, there are various sources of natural acids that can be explored as local raw materials, one of which is palm sap vinegar and lime juice extract (Masyitaha *et al.*, 2017).

Lontar sap vinegar is a fermented product from sap produced by Lontar palm (*Borassus flabellifer*). In several areas in Indonesia, palm sap vinegar has long been used as a food preservative (Bora & Gasong, 2021) and has an acetic acid content of between 3.68%–13.86% (Rahmawati *et al.*, 2021), so it has the potential to be used in gelatin production. In addition, palm vinegar is a local product that is easily obtained and cheap, so it can be an economical alternative in the gelatin processing industry (Li *et al.*, 2021). Meanwhile, lime (*Citrus aurantiifolia*) is a fruit that is very rich in citric acid. Lime juice extract has been used in various food processing applications such as a natural acidifying agent, with a citric acid content ranging from 7% to 7.6% by weight (Nurvita *et al.*, 2015) so that it has the potential to be used in the production of fish gelatin (Lestari *et al.*, 2024).

Research on the use of natural acids in the production of fish gelatin has shown mixed results. Several studies have found that the use of natural acids can produce gelatin with good physical and chemical quality, although sometimes the efficiency is slightly lower than HCl. For example, Arnamalia *et al.* (2021) reported that the use of citric acid for the extraction of gelatin from white snapper (*Lates calcarifer*) skin produced good functional properties, such as having a competitive gel strength compared to commercial gelatin. The yield of gelatin produced using HCl and organic acids such as citric acid had relatively comparable values, namely 10.58% gelatin at a HCl concentration of 5% and 9.74% at a citric acid concentration of 9% (Lamalelang *et al.*, 2019). On the other hand, research by Masyitaha *et al.* (2017), showed that the use of vinegar as a soaking acid for tuna fish bones produced gelatin with a yield and chemical characteristics that were almost equivalent to gelatin produced with HCl.

The use of these two ingredients as alternatives to natural acids in the production of skipjack tuna bone gelatin has not been widely explored. In fact, Indonesia has great potential in developing this local raw material, considering the availability of palmyra palm and lime trees that are widely spread in various regions (Priyosetyoko *et al.*, 2022). Research on the use of palm sap vinegar and lime juice extract in the production of gelatin from skipjack tuna bones has never been conducted. Therefore, this study aims to explore the effectiveness of palm sap vinegar and lime juice extract at various concentrations as a substitute for HCl in the production of skipjack tuna bone gelatin. The results of this study are very useful in the use of more environmentally friendly organic acids.

2. MATERIALS AND METHODS

2.1. Research Material

This study uses skipjack tuna fish bones as the main raw material in gelatin production. Fish bones were obtained from PT MAS located in the Tenau Kupang Port area which is engaged in producing fish fillets for export. Skipjack tuna was chosen because of its abundant availability in Indonesian waters and its potential as a source of collagen that can be hydrolyzed into gelatin. The fish bones used were obtained from waste from the fish processing industry in coastal areas. In addition, palm sap vinegar and lime juice extract were used as local acid compounds that function as substitutes for hydrochloric acid (HCl), which is commonly used in gelatin production. Palm sap vinegar is produced through fermentation of Lontar sap, fresh palm sap is obtained from tappers and then fermented for 30 days. Meanwhile, lime juice extract is obtained by extracting juice from lime fruit, by cutting fresh lime fruit and squeezing it to get the juice. Limes are obtained from farmers who specifically cultivate citrus. HCl was used as a control in this study, which was obtained from the Agricultural Product Technology Laboratory of the Kupang State Agricultural Polytechnic. Other chemicals used included buffer solutions and reagents for chemical analysis of gelatin, such as determining water, ash, fat, protein, and pH content.

2.2. Research Design

This study used an experimental design with a 3 x 3 factorial design. The first factor was the type of acid consisting of three types: A0 = HCl (control), A1 = palm sap vinegar, and A2 = lime juice extract. The second factor was the acid concentration (in % volume) with three levels, namely K1 = 5%, K2 = 7%, and K3 = 9%. So there were a total of nine treatment combinations that would be tested in this study. Each treatment combination would be repeated three times to ensure the validity of the results.

2.3. Research Implementation Stages

The stages of this research begin with the preparation of raw materials, namely the collection of Skipjack tuna bones from the fish processing industry. The bones are then boiled for 60 minutes at a temperature of 80°C, then cleaned of remaining meat and reduced in size, then washed three times with running water until clean. After that, the fish bones are soaked in an acid solution according to the treatment, namely palm vinegar, lime juice extract, and HCl at different concentrations (5%, 7%, and 9%). Soaking is carried out for 48 hours at room temperature until they become olean (soft bones). The olean is washed with distilled water until pH 7. The olean is then put into an Erlenmeyer flask and distilled water is added with a ratio of 1:1.5 (one kg of olean to 1.5 liters of distilled water) and put in a water bath with a heating temperature of 60°C for 7 hours to produce a collagen solution. Well-hydrolyzed collagen from fish bones can be recognized by several physical characteristics, namely first, the color of the solution is clear and slightly yellowish. Second, the aroma, which has a distinctive aroma, a slight smell of broth or meat. If the hydrolysis is not perfect, there will be a fishy or unpleasant odor due to the remaining protein that is not hydrolyzed.

After extraction, the gelatin solution is filtered with a filter cloth to separate insoluble solids, then dried using an oven at a temperature of 60°C until the resulting gelatin is in the form of dry sheets. The baking time ranges from 6–12 hours, depending on the water content in the extracted gelatin solution. The gelatin sheets are then crushed into powder using a blender for further analysis.

2.4. Research Variables and Data Analysis

The variables measured in this study include: gelatin yield (%), calculated based on the comparison of the weight of the gelatin produced with the weight of the initial dry skipjack tuna bones. Chemical quality parameters, including: gelatin pH (pH meter), water content (%) using the drying oven method, ash content (%) was determined using a furnace, fat content (%) using the Soxhlet extraction method, and protein content (%) was carried out using the Kjeldahl method. The data obtained from this study were analyzed statistically using the analysis of variance (ANOVA) method to see the significant effect of each factor (type of acid and acid concentration) on the yield and chemical quality of gelatin. If there is a significant effect, further testing is carried out using Duncan Multiple Range Test (DMRT) to see the differences between treatments. The best treatment is seen from the value of the variable that

is close to or in accordance with the SNI for gelatin quality. All analyses were carried out using SPSS statistical software version 26. In addition, the results of the analysis will also be compared with previous literature and research to provide a more in-depth discussion of the effectiveness of palm vinegar and lime juice extract as a substitute for HCl in the production of fish bone gelatin.

3. RESULTS AND DISCUSSION

3.1. Skipjack Tuna Bone Gelatin Yield

The results of the variance analysis showed that there was no significant interaction (p -value $0.125 > 0.05$) between the type of acid compound and the acid concentration on the gelatin yield. However, each single factor, both the type of acid and the acid concentration, had a significant effect (p -value $0.002 < 0.05$), indicating that both the type of acid compound used and the acid concentration applied had a separate direct impact on the yield of Skipjack Tuna bone gelatin. This indicates that these factors independently affect the gelatin yield, but there is no evidence that the interaction between the two factors worsens or increases the effect of each other. The average results of the DMRT test of Skipjack Tuna bone gelatin yield are presented in Table 1.

Table 1. Average yield of gelatin from skipjack tuna bones under the influence of acid type and concentration treatments

Treatment	Yield (%)
Type of Acid Compound	
HCl	6.60a $\pm$ 0.92
Palm Sap Vinegar	6.38a $\pm$ 0.80
Lime Extract	5.79b $\pm$ 0.61
Acid Concentration	
5% volume	5.49c $\pm$ 0.21
7% volume	6.24b $\pm$ 0.31
9% volume	7.14a $\pm$ 0.62

Note: Mean values followed by the same letter indicate no significant difference based on the DMRT test at a 5% significance level. Values are means followed by " $\pm$ " standard deviation (SD).

Based on the results of the DMRT further test at a significance level of 5% (Table 1), the average gelatin yield from the acid compound type treatment showed that the use of HCl as a control gave the highest yield with a value of 6.60%. This is consistent with previous studies stating that hydrochloric acid is effective in hydrolyzing collagen and producing gelatin with high yield (Trilaksani *et al.*, 2012). The gelatin yield produced by palm sap vinegar reached 6.38%, which was not significantly different from the HCl yield at the 5% level. This shows that palm sap vinegar, as a local acid compound, has good potential in producing gelatin with a yield that is almost equivalent to HCl. Meanwhile, lime juice extract produced a lower yield, which was 5.79%, which was statistically significantly different from HCl and palm sap vinegar. The gelatin yield produced by lime was lower than that of HCl and palm sap vinegar, due to the less efficient collagen breakdown process. This is because citric acid is weaker in lowering pH compared to hydrochloric acid (HCl) or palm sap vinegar. Citric acid, although acidic, has a lower ionization strength, so it is unable to damage the peptide bonds in collagen with the same efficiency. In contrast, HCl and palm sap vinegar have a higher acidity level, which allows for more effective hydrolysis of collagen into gelatin (Arnamalia *et al.*, 2021).

In addition to the type of acid compound, the acid concentration treatment also has a significant effect on the gelatin yield. The DMRT test results showed that the gelatin yield increased with increasing acid concentration. At an acid concentration of 5%, the gelatin yield produced was 5.49%, which was significantly lower than the concentrations of 7% and 9%. At a concentration of 7%, the gelatin yield increased to 6.24%, and at a concentration of 9%, the yield reached the highest value, which was 7.14%. The increase in yield along with the increase in acid concentration is due to the increasing ability of the acid to hydrolyze collagen bonds in fish bones, so that more collagen is broken down into gelatin (Arshad *et al.*, 2021).

However, although higher acid concentrations produce higher yields, it should be noted that excessive increases in acid concentrations can risk damaging the functional properties of gelatin, such as viscosity and gel strength, due to

excessive degradation of the collagen chain (Masyitah *et al.*, 2017). Although a concentration of 9% provides the highest yield, other aspects of gelatin quality must also be considered in choosing the optimal acid concentration. Based on the gelatin yield produced, this study shows that palm sap vinegar has great potential as an alternative organic acid to replace HCl in gelatin production, especially at higher concentrations, such as 9%. The use of lime juice extract, although it produces a lower yield, can also be a more environmentally friendly alternative compared to HCl, especially in the context of sustainable fishery product processing in Indonesia (Rahman *et al.*, 2022).

3.2. Chemical Quality of Skipjack Tuna Bone Gelatin

In general, the results of the variance analysis on the quality of Skipjack Tuna bone gelatin, which include: water content, pH, ash content, fat content, and protein content, show that there is no significant interaction between the type of acid compound and acid concentration on the quality of gelatin. However, each single factor, both the type of acid compound and the acid concentration, have a significant effect. This shows that both the type of acid used and the acid concentration applied have a direct impact on the quality of Skipjack Tuna bone gelatin. The average results of the DMRT test on the chemical quality of skipjack tuna bone gelatin are presented in Table 2.

Table 2. Average chemical quality of gelatin from skipjack tuna bones under the influence of locally sourced acid compounds and concentration treatments

Treatment	Moisture Content (%)	pH	Ash Content (%)	Fat (%)	Protein (%)
Type of Acid Compound					
HCl	11.27b $\pm$ 1.04	4.97a	2.42a	1.84a	53.93a $\pm$ 2.29
Palm Sap Vinegar	11.66b $\pm$ 1.75	5.08a	2.45a	1.78a	53.77a $\pm$ 2.72
Lime Extract	13.12a $\pm$ 0.92	4.66b	2.28b	1.60b	48.87b $\pm$ 3.07
Acid Concentration					
5% volume	13.76a $\pm$ 0.76	5.32a	1.97b	1.53b	49.00b $\pm$ 3.13
7% volume	11.76b $\pm$ 1.32	4.89b	2.43a	1.95a	52.74a $\pm$ 2.19
9% volume	10.89b $\pm$ 1.09	4.49b	2.75a	1.84a	54.82a $\pm$ 2.54

Note: Mean values followed by the same letter in the same column indicate no significant difference based on the DMRT test at a 5% significance level. Values are means followed by " $\pm$ " standard deviation (SD).

3.2.1. Water Content of Gelatin

Water content is one of the important parameters in determining the quality of gelatin, which affects storage stability, functional properties, and texture of the final product. The results of the variance analysis showed that there was no significant interaction (p -value $0.08 > 0.05$) between the type of acid compound and acid concentration on the water content of gelatin. However, each single factor, both the type of acid compound and the acid concentration, had a significant effect (p -value $0.023 < 0.05$) on the water content of gelatin.

The results of the DMRT test showed that the use of HCl as a control produced gelatin with a lower water content, namely 11.27%, and was not significantly different from palm sap vinegar which gave a water content of 11.66%. The low water content in gelatin extracted using HCl and palm sap vinegar indicates that these acids are more effective in reducing water content during the gelatin extraction process, possibly due to their effect in breaking down collagen bonds more efficiently, resulting in a more compact gelatin structure (Sutardi *et al.*, 2020). On the other hand, the use of lime juice extract produced gelatin with the highest water content, which was 13.12%. This indicates that gelatin extracted with citric acid from lime absorbs more water during the extraction process. This could be due to the effect of citric acid on the gelatin structure, which tends to be more hydrophilic and has a higher affinity for water molecules (Arnamalia *et al.*, 2021).

The acid concentration tested in this study also had a significant effect on the water content of gelatin. The results of the DMRT test showed that an acid concentration of 5% produced gelatin with the highest water content, which was 13.76%, which was significantly different from the concentrations of 7% and 9%. The higher water content at a concentration of 5% indicates that at lower concentrations, the acid is unable to optimally break down the collagen structure, so that the resulting gelatin structure is looser and tends to absorb more water (Masyitah *et al.*, 2017). When the acid concentration was increased to 7% and 9%, the water content of fish bone gelatin decreased, by 11.76% and

10.89%, respectively. This is because acid with a high concentration is more effective in breaking down the triple-helix structure of collagen, producing gelatin with a denser molecular network. This condition causes the gelatin's capacity to hold water (water-holding capacity) to decrease, so that the water content in the final product becomes lower (Priyosetyoko *et al.*, 2022). In addition, acid with a high concentration can also increase the rate of water removal during the drying process, because the denser gelatin structure makes it easier to release water. This mechanism is in line with previous findings showing that acid processing conditions affect the texture and physical properties of gelatin (Johnston-Banks, 1990).

Although lower water content is generally considered better in terms of storage stability, it is important to note that too low a water content can affect the functional properties of gelatin, such as solubility and viscosity. Therefore, a balance between water content and the functional properties of gelatin must be considered in the production process. In the context of this study, acid concentrations of 7% and 9% provided optimal water content with values close to the standard commercial gelatin water content, which is between 8-12% (Arshad *et al.*, 2021).

3.2.2. pH of Gelatin

The results of the variance analysis showed that there was no significant interaction (p -value $0.102 > 0.05$) between the type of acid compound and the acid concentration on the pH of gelatin. However, each single factor, both the type of acid compound and the acid concentration, had a significant effect (p -value $0.009 < 0.05$) on the pH of gelatin. The results of the DMRT further test showed that the use of HCl produced gelatin with the lowest pH, namely 4.97, but it was not significantly different from the pH of gelatin produced from the use of palm sap vinegar, which had a pH of 5.08. This shows that these two acid compounds have almost the same ability to break down collagen, but with a slight difference in effect on the acidity of the resulting gelatin (Trilaksani *et al.*, 2012). On the other hand, the use of lime juice extract produced gelatin with a lower pH, namely 4.66, which was significantly different from the other two treatments. This can be explained by the citric acid content (7%) in the lime juice extract, which contributes to the higher acidity of the final product (Li *et al.*, 2021; Arnamalialia *et al.*, 2021).

The treatment of acid concentration (% volume) also significantly affected the pH of gelatin. Based on the results of the DMRT test, a 5% acid concentration produced gelatin with the highest pH of 5.32, which was significantly different from a 7% concentration which produced a pH of 4.89. This occurs because a higher acid concentration increases the amount of hydrogen ions (H^+) available in the solution. These hydrogen ions lower the pH of the solution by suppressing the ionization level of amino acid residues in collagen during the hydrolysis process. As a result, the resulting gelatin has a lower pH. These results are also in line with previous studies that reported that the use of high concentrations of acid during the extraction process lowers the pH of gelatin because collagen residues undergo more intensive ionization in an acidic environment (Jongjareonrak *et al.*, 2006; Arshad *et al.*, 2021). However, it should be noted that too low a gelatin pH, such as in lime extract or high acid concentrations, can affect the functional properties of gelatin such as solubility and gel strength, which may be less desirable in certain applications (Maszytah *et al.*, 2017; Li *et al.*, 2021).

In the context of commercial gelatin standards, the pH of gelatin usually ranges from 4.5 to 6.5 depending on the source of raw materials and the process used. The results of this study indicate that all treatments are still within the appropriate pH range for commercial gelatin, although the use of lime extract produces a pH that tends to be lower. These lower pH gelatins may be more suitable for applications where acidic properties are more desirable, such as in certain acidic food products or pharmaceuticals (Priyosetyoko *et al.*, 2022).

3.2.3. Ash Content of Gelatin

The results of the variance analysis showed that there was no significant interaction (p -value $0.130 > 0.05$) between the type of acid compound and acid concentration on the ash content of gelatin. However, each single factor, both the type of acid compound and the acid concentration, had a significant effect (p -value $0.009 < 0.05$) on the ash content of gelatin. The results of the DMRT test showed a significant difference between the treatments of acid type and concentration on the ash content. The use of HCl produced an ash content of 2.42%, which was not significantly different from the ash content produced by the use of palm vinegar, which was 2.45%. Both of these treatments provided a higher ash content compared to the use of lime juice extract, which produced an ash content of 2.28%

(Masyitah *et al.*, 2017). The low ash content in the treatment with lime juice extract can be caused by the citric acid content which is more capable of dissolving mineral components in fish bones, so that the gelatin obtained is cleaner from mineral residues (Arnmalia *et al.*, 2021). On the other hand, the use of HCl and palm sap vinegar gave almost equivalent results in breaking down mineral components, with insignificant differences in the ash content of the gelatin produced (Jongjareonrak *et al.*, 2006).

In addition to the type of acid compound, the acid concentration (% volume) also had a significant effect on the ash content of gelatin. Based on the results of the DMRT test, an acid concentration of 5% produced the lowest ash content of 1.97%, which was significantly lower than the concentrations of 7% and 9%. At a concentration of 7%, the ash content of the gelatin produced was 2.43%, while a concentration of 9% produced the highest ash content of 2.75%. The higher the acid concentration, the greater the ability of the acid to dissolve mineral components because the acid produces more hydrogen ions (H^+) in solution (Sutardi *et al.*, 2020). However, increasing the acid concentration does increase the ability to dissolve minerals so that the ash content increases, but under certain conditions, the amount of minerals left behind can also increase (Priyosetyoko *et al.*, 2022). This can happen because the reaction between acid and minerals is not always perfect. At very high acid concentrations, mineral dissolution can be hampered by the formation of a layer of salt or new compounds on the surface of the mineral, such as calcium phosphate or calcium sulfate, which are less soluble. This layer functions as a physical barrier, so that H^+ ions from the acid have difficulty penetrating and continuing deeper dissolution.

In general, the ash content of gelatin obtained from this study is within the range that corresponds to commercial gelatin standards, which usually have an ash content below 3%. However, lower ash content, such as that obtained at 5% acid concentration and the use of lime juice extract, is more desirable to produce better quality and purer gelatin (Arshad *et al.*, 2021). Lower ash content indicates that the gelatin product has less mineral content, which is important for applications in the food and pharmaceutical industries, where product purity is of great importance.

3.2.4. Fat Content of Gelatin

The results of the variance analysis showed that there was no significant interaction ($p\text{-value } 0.086 > 0.05$) between the type of acid compound and acid concentration on the fat content of gelatin. However, each single factor, both the type of acid compound and the acid concentration, had a significant effect ($p\text{-value } 0.002 < 0.05$) on the fat content of gelatin. The results of the DMRT test showed that there was a significant difference between treatments on the fat content of gelatin. The use of HCl and palm sap vinegar produced a relatively higher fat content and was not significantly different (1.84% and 1.78%), compared to the use of lime juice extract, which produced a lower fat content of 1.60%.

The low fat content in the treatment with lime juice extract was caused by the ability of citric acid to be more effective than HCl and palm sap vinegar in breaking down and dissolving fat during the fish bone extraction process. This is because of its nature as a natural emulsifying agent. Citric acid has a carboxyl group ($-COOH$) that can bind metal ions and form complexes with fat molecules, thereby breaking down fat into a more water-soluble form. In addition, the chelating agent properties of citric acid help dissolve fat-bound compounds, such as calcium, which usually block the release of fat from bone tissue. As a result, the gelatin produced from citric acid treatment is cleaner from residual fat (Arnmalia *et al.*, 2021).

In addition to the type of acid compound, the acid concentration treatment (% volume) also showed that an acid concentration of 5% produced the lowest fat content, namely 1.53%, and was significantly different from the concentrations of 7% and 9% with gelatin fat content of 1.95% and 1.84%, respectively. Increasing the acid concentration to 7% and 9% significantly increased the fat content of fish bone gelatin. This is because acid at high concentrations not only dissolves fat but also breaks down the collagen protein structure excessively. This can cause the release of fat compounds that were previously trapped in the protein matrix to become more mixed in the gelatin solution. In addition, acid that is too concentrated can reduce the effectiveness of its emulsion, so that the fat is not completely separated from the solution. As a result, some fat remains and is bound in the resulting gelatin (Alves *et al.*, 2022; Priyosetyoko *et al.*, 2022).

In general, the fat content of gelatin obtained from this study is still within reasonable limits and close to commercial gelatin standards, which usually have a fat content below 2% (BSN, 1995). However, gelatin with a lower fat content, such as that produced at an acid concentration of 5% and with the use of lime juice extract, is more desirable in applications that require gelatin with a high level of purity, such as in the food and pharmaceutical industries. Gelatin products with lower fat content tend to be more stable during storage and are not easily oxidized which can cause changes in aroma and taste (Arshad *et al.*, 2021; Wong & Tan, 2017).

3.2.5. Protein Content of Gelatin

The results of the variance analysis showed that there was no significant interaction (p -value $0.125 > 0.05$) between the type of acid compound and acid concentration on the gelatin protein content. However, each single factor, both the type of acid compound and the acid concentration, had a significant effect (p -value $0.002 < 0.05$) on the gelatin protein content. The results of the DMRT test showed that there was a significant difference between treatments on the gelatin protein content. The HCl and palm vinegar treatments produced the highest protein content (53.93% and 53.77%) and were not significantly different. This shows that both types of acid compounds have good and equal ability to break down collagen to produce gelatin with a high protein content. Meanwhile, the use of lime juice extract produces gelatin with a lower protein content, namely 48.87% (Masyitah *et al.*, 2017). The low protein content in the treatment with lime juice extract can be caused by citric acid which is milder in breaking down peptide bonds in collagen (Li *et al.*, 2021) compared to HCl and acetic acid contained in palm vinegar, so that the amount of collagen hydrolyzed into gelatin is lower (Pertiwi *et al.*, 2018; Arnamalia *et al.*, 2021).

In addition, the acid concentration treatment (% volume) also significantly affected the protein content of gelatin. Increasing the acid concentration to 7% and 9% significantly increased the protein content of fish bone gelatin (54.82%, and 49%) higher than the concentration of 5%, which produced a protein content of 49.00%. This is because acid at high concentrations is more effective in breaking bonds between collagen protein and other components, such as minerals and fats, resulting in gelatin extract with a purer protein content. Acid at high concentrations can also accelerate the hydrolysis of peptide bonds, which releases more soluble protein fragments into the solution. As a result, the resulting gelatin has a higher protein content (Sompie *et al.*, 2015; Alves *et al.*, 2022; Ismail & Hmid, 2020). This is in line with the research of Prasetyo *et al.* (2023), that treatment with higher acid concentrations increases the efficiency of collagen protein extraction without damaging the structure.

Overall, the results of this study indicate that the use of HCl and palm vinegar can produce gelatin with high and almost equivalent protein content. Lime juice extract, although it produces gelatin with a lower protein content, still produces a protein content that is good enough to meet commercial gelatin standards. In terms of acid concentration, higher concentrations such as 9% give the best results in terms of protein content, which means that the collagen hydrolysis process is more efficient at higher concentrations. However, it should be noted that in addition to protein content, other factors such as solubility, viscosity, and other physical properties of gelatin also need to be taken into account in determining the optimal concentration for commercial gelatin production (Arshad *et al.*, 2021).

In general, the gelatin produced in this study meets the protein standards for commercial gelatin, where the protein content of gelatin is usually in the range of 40-90% (BSN, 1995), depending on the source of raw materials and the extraction method used. Higher protein content indicates that the resulting gelatin has good functional properties, such as high gel strength and better solubility, which are desirable characteristics in food and pharmaceutical applications (Sompie *et al.*, 2015; Alves *et al.*, 2022).

4. CONCLUSION

Based on the results, it can be concluded that the best type of acid treatment for the extraction of skipjack tuna bone gelatin is palm sap vinegar. Palm sap vinegar has a performance equivalent to hydrochloric acid (HCl) in producing high gelatin yield and quality, even better than citric acid derived from lime extract. While the acid concentration treatment, the best results were obtained at acid concentrations of 7% and 9%. At this concentration, the acid works optimally to break down peptide bonds in collagen and increase the yield and quality of skipjack tuna bone gelatin extraction. Based on results of this research, it is recommend to use palm sap vinegar as a more environmentally

friendly and effective source of acid in the extraction of skipjack tuna bone gelatin, with a concentration of 7% to 9%. The use of this acid concentration can optimize both the yield and quality of the gelatin produced, so that it can be used for various industrial applications, such as raw materials in the manufacture of food, cosmetic, and pharmaceutical products.

ACKNOWLEDGEMENTS

Gratitude is expressed to the Center for Research and Community Service of the Kupang State Agricultural Polytechnic, which has funded this research through the Applied Research scheme FY 2024.

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